Remarks

Claim 2 has been cancelled and is being replaced by new claim 25. New claim 25 is believed to conform more closely to the preferred language set forth in the Office Action and discussed in the telephone interview. Newly added claims 26 through 33 are dependent thereon. Support for claims 25 through 27, 34 and 35 is found in the specification, page 9 line 29 through page 10 line 9. Support for newly added claims 28 through 33, and 36 through 39 is found on page 12, lines 3 through 15.

Objections under 35 USC § 112

The title of the specification was objected to as not being descriptive; applicants have amended the title as discussed during the telephone interview. The title as amended is believed to more accurately describe the presently claimed invention; accordingly, applicants request that the objection be withdrawn.

The disclosure was further objected to for including several informalities, including misspelling amino acid on page 5, inclusion of an extraneous period on page 10, and misspelling 'CYLD' on page 13. Applicants have corrected these obvious errors, and request that this objection be withdrawn.

The disclosure was also objected to for the inclusion of an embedded hyperlink on page 16. Applicants have reformatted the information presented such that it should not appear as an embedded hyperlink in an electronic version of the specification, and request that this objection be withdrawn.

During the telephone interview, it was noted that the specification contained a typographical error in numbering of the amino terminal residues of specific fragments in the first paragraph on page 10, namely in the listing of a supposed residue 3843 (which actually refers to residue 383). Applicants respectfully submit that this is an obvious typographical error, and would immediately be understood as such by one of ordinary skill in the art, for at least three reasons: its place in the numerical listing of amino termini (i.e., between 384 and 382), the fact that there are only 419 amino acids in the NEMO

polypeptide as shown in SEQ ID NO:2 (thus there is no amino acid 3843 with which it might be confused), and by the preceding discussion on page 10 of the specification wherein it is noted that useful fragments of the NEMO polypeptide include the region from about amino acid 387 to 419 and additional fragments thereof that bind CYLD and are truncated by about five to ten amino acids.

Applicants have also amended this paragraph to include a comma between residues 380 and 379, which is believed to be an obvious typographical error and subject to correction for the reasons stated above. Inasmuch as these changes correct errors that would be identified as obvious by one of ordinary skill in the art and thus do not constitute new matter, applicants respectfully request that the amendment to the specification be entered.

Rejections under 35 USC §§ 101 and 112

Claim 2 was rejected under 35 USC § 101 as allegedly not being supported by a specific, substantial, and credible utility, or in the alternative a well established utility. The claim was also rejected under 35 USC § 112, first paragraph, as one of ordinary skill in the art would allegedly not know how to use the claimed invention. Applicants respectfully disagree; claim 2 has been canceled, and is being replaced herein by newly added claim 25. The differences between canceled claim 2 and new claim 25 are not such as to alter the scope of what applicants regard as their invention, but merely serve to clarify the language of the claim.

Applicants respectfully assert that the utility of the presently claimed methods is asserted explicitly throughout the specification, for example at page 2, lines 29 and 30, wherein it states that the present invention provides methods for screening for a molecule that antagonizes (or agonizes the activity, an allegedly separate invention that has been restricted from the present application) the activity of NEMO in CD40 signaling. Inhibition of the activity of NEMO in CD40 signaling will decrease or downregulate biological activities of CD40, including deleterious effects of CD40-mediated immune or inflammatory response (including atherosclerosis, arthritis, multiple sclerosis (MS),

systemic lupus erythematosous (SLE), thrombosis, graft versus host disease and/or graft rejection (paragraph spanning pages 2 and 3 of the specification).

Moreover, applicants further submit that the asserted utility of the presently claimed invention is specific, substantial and credible. The Manual of Patent Examining Procedure (MPEP), in section 2107.01, General Principles Governing Utility Rejections, discusses and applies the terms specific, substantial and credible as they apply to utility. In discussing "specific" with regards to utility, the MPEP states:

> "A general statement of diagnostic utility, such as diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed. Contrast the situation where an applicant discloses a specific biological activity and reasonably correlates that activity to a disease condition. Assertions falling within the latter category are sufficient to identify a specific utility for the invention." (emphasis added)

In discussing "specific" with regards to utility, the MPEP states:

> "A "substantial utility" defines a "real world" use. .... For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring." (emphasis added)

With regard to the credibility of utility for therapeutic or pharmacologic agents, the MPEP notes that:

> "Courts have repeatedly found that the mere identification of a pharmacological activity of a compound that is relevant to an asserted pharmacological use provides an "immediate benefit to the public" and thus satisfies the utility requirement. As the Court of Customs and Patent Appeals held in *Nelson v. Bowler*:
>
> > Knowledge of the pharmacological activity of any compound is obviously beneficial to the public. It is inherently faster and easier to combat illnesses and alleviate symptoms when the medical profession is armed with an arsenal of chemicals having known pharmacological activities. Since it is crucial to provide researchers with an incentive to disclose pharmacological activities in as many compounds as possible, we

> conclude that adequate proof of any such activity constitutes a showing of practical utility.
> *Nelson v. Bowler*, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980)."
> (emphasis in original)

Accordingly, the presently claimed methods of identifying compounds that antagonize a specific activity that is involved in specific disease states invention provide immediate benefit to the public, and have a real-world use that is specific, substantial and credible.

However, even if applicants had not asserted a utility for any compounds identified by using the presently claimed methods, applicants submit that it is known in the art that it is desirable to seek antagonists of CD40, as shown by the publication of articles discussing investigations done (including human clinical trials) on several CD40 antagonists. Applicants are including herewith the abstracts of nine such articles, (Exhibit A), discussing three CD40 antagonists (two antibodies that bind CD40L, termed IDEC-131 and 5C8, and one antagonistic antibody that binds CD40, referred to as 5D12). The abstracts are believed to provide sufficient evidence that there is an established utility for CD40 antagonists, however, applicants will provide copies of the full-length publications if the Examiner so desires.

Thus, the utility of the presently claimed invention is specific, substantial and credible, and the application conforms with the requirements of 35 USC §§ 101 and 112 for utility and enablement with regards to using the invention. Applicants respectfully request that the rejections for lack of utility be withdrawn.

Rejections under 35 USC § 112, first paragraph

Claim 2 was rejected under 25 USC § 112, first paragraph, as not being enabled because the specification allegedly does not reasonably provide enablement for methods utilizing fragments or variants of NEMO and CYLD polypeptides. The Examiner asserts that it would constitute undue experimentation to practice the claimed invention due to the alleged unpredictability of knowing the degree of variance allowed. Claim 2 has been canceled, and is being replaced with claim 25, which states that fragments and variants of the states polypeptides are at least 80% identical to the polypeptides set forth in the

Sequence Listing, and retain the ability to bind the respective binding partner (i.e., NEMO fragments or variants are capable of biding CYLD, and CYLD fragments or variants are capable of binding NEMO). Applicants respectfully submit that it is a matter of routine experimentation to make such fragments or variants, evaluate whether they are at least 80% identical to the native polypeptides, and determine whether they are capable of binding the other binding partner. Accordingly, this claim is believed to address the Examiner's rejection, and applicants respectfully request that the rejection be withdrawn.

Rejections under 35 USC § 112, first paragraph

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly being vague and indefinite in the use of an abbreviation. Applicants respectfully disagree; the abbreviations would be well-understood by those of skill in the art. Moreover, claim 2 has been canceled, and is being replaced by claim 25, which spells out the full names of the polypeptides. This amendment is believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly unclear in the use of the phrase "binding of NEMO and CYLD." According to the Examiner, it unclear whether this phrase denoted binding of NEMO to CYLD, or binding of NEMO and CYLD to other compounds. Applicants respectfully disagree; those of skill in the art would understand the phrase to denote binding of NEMO to CYLD, as disclosed in the specification. Moreover, claim 2 has been canceled, and is being replaced by claim 25, which recites a method for identifying a compound that inhibits binding of NEMO to CYLD. Newly added independent claim 34 also uses this phrasing, which is believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly vague and indefinite in the use of the phrase "comprising amino acids 287 thorough 419 [of] SEQ ID NO:2." According to the Examiner, it unclear whether the claim is referring to the entire portion of residues 287 though 419, or just a section within it. Applicants respectfully assert that the word 'comprising' is synonymous with containing, and as such

is inclusive (that is, it includes the recited characteristics). Moreover, the phrase also includes the word 'through' with respect to the amino acid sequence, meaning that all of the amino acids between the stated end-points are included. In fact, applicants have included fragment language in the claims, differentiating such fragments from the longer polypeptides.

Additionally, it is well-known in the art (and set forth in the specification) that a number of moieties that can be added to polypeptides to yield conjugates, such as fusion proteins. Fusion proteins can comprise the peptide set forth in the claims and a tag peptide that facilitates detection and/or purification. The use of 'comprising' further allows use of the full length-polypeptide (i.e., full-length NEMO or CYLD), as well as polypeptides having random or specific amino acids appended to either the amino terminus, the carboxy terminus or both, which are within the ability of one in the ordinary skill in the art to produce.

During the telephone interview, a concern was stated that the use of the transitional phrase 'comprising' might be construed as reading on polypeptides that comprised sufficient extra residues that they no longer exhibited the desired characteristic (i.e., NEMO polypeptides that were so bulky or folded in such a way as to no longer bind CYLD, and vice-versa). Claim 2 has been canceled, and is being replaced by claim 25, which states that NEMO polypeptides useful in the presently claimed invention have the ability to bind CYLD polypeptides, and that CYLD polypeptides useful in the presently claimed invention have the ability to bind NEMO polypeptides. Newly added independent claim 34 uses the phrase 'consisting essentially of,' indicating that the scope of the claim includes the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in original).

This phrasing is believed to address the concerns brought up in the telephone interview and the Office Action; the present claims do not read on inoperative embodiments that might have been construed as being comprehended within the scope of the claims. As such, the revised phrasing in the claims, while more particularly pointing out and distinctly claiming what applicants believe to be their invention, does not narrow

the scope of the claims. Applicants respectfully assert their right to pursue all remedies available against potential infringers, including use of the doctrine of equivalents. The present claims are believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

A related concern with respect to the use of the word 'comprising' that was discussed during the telephone interview was whether this transitional phrase allows comprehension within the scope of the claims of polypeptides that have additional moieties inserted between the termini specified in the claims. Applicants note that phrases such as "comprising amino acids a through z of SEQ ID NO:X" are widely used in the patent field, and are understood by those of ordinary skill in the art to be directed to polypeptides having a particular primary structure, namely a specified sequence of amino acids (including functional equivalents thereof, for example, variants as discussed in the present specification). In fact, a search of the USPTO database of issued US patents for the phrase "comprising amino acid" in the claims yielded over 3,000 issued patents that use this phrase, including patents issued as recently as this month and as long ago as 1978.

Applicants respectfully submit that the language of the claims must be interpreted in light of the teachings of the specification and that which is well-known in the art. To construe this long-known and well-understood phrase to read on polypeptides into which have been intercalated moieties that significantly alter the structure and function of the polypeptide would engender a great deal of uncertainty in the interpretation of not only the present claims, but those found in numerous issued patents. The Examiner is invited to apprise applicants of recent changes in patent law that alter the meaning of this phrase and related phrases, and thereby necessitate changing long-standing, art-accepted practice in describing and claiming polypeptides.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly being vague and indefinite in the use of the phrase "according to." Applicants respectfully disagree; the phrase would be well-understood by those of skill in the art. Moreover, claim 2 has been canceled, and is being replaced by claim 25, which does not use the phrase 'according to' in the manner rejected by the Examiner. The newly added claims

are believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly being vague and indefinite in the use of the phrase "capable of binding." According to the Examiner, it is unclear what the phrase means as far as the amount of binding required. Applicants respectfully disagree; the phrase is well-understood by those of skill in the art: a NEMO polypeptide capable of binding a CYLD polypeptide is one that will bind CYLD when the two are brought into contact (in the absence of an inhibitor thereof). Moreover, claim 2 has been canceled, and is being replaced by claim 25, which specifies that a compound identified as an inhibitor of the binding of NEMO to CYLD is antagonist of NEMO activity in CD40 signaling. New claim 34 is similarly phrased. The present claims are believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly being vague and indefinite in the use of the word "variants." Claim 2 has been canceled, and is being replaced by claim 25, which specifies that variants within the scope of the claims are those that are at least 80% identical to the native polypeptide and that retain the ability to bind the binding partner (i.e., NEMO variants are able to bind CYLD and CYLD variants are able to bind NEMO). New claim 34 is similarly phrased. The present claims are believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

Claim 2 was rejected under 25 USC § 112, second paragraph, as allegedly being vague and indefinite in the use of the phrase "inhibits the binding activity." According to the Examiner, it is unclear to what degree the activity is inhibited. Claim 2 has been canceled, and is being replaced by claim 25, which specifies that a compound identified as an inhibitor of the binding of NEMO to CYLD is one that inhibits binding of these two polypeptides to each other by at least 50%. New claim 34 is similarly phrased. The present claims are believed to address the Examiner's rejection; accordingly, applicants request that the rejection be withdrawn.

CONCLUSIONS

Claims 25 through 39 are now pending in the application and are believed to be in condition for allowance. Applicants have incorporated language suggested by the Examiner, to speed prosecution and allowance of the claims. The amendments or changes in phraseology made herein do not alter the scope of the claims from that of originally filed claim 2. Accordingly, applicants respectfully assert their right to pursue all remedies available against potential infringers, including use of the doctrine of equivalents. If the examiner has any questions or concerns about the present claims, she is asked to contact the undersigned at the direct dial number given below, to facilitate prosecution and speed allowance of the claims.

Applicants believe no fees are due, however the Commissioner is authorized to charge any necessary fee, or credit any overpayment, to Deposit Account No. 09-0089.

Immunex Corporation
Law Department
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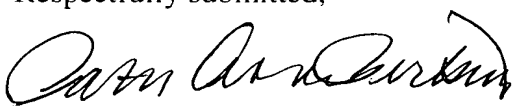
Respectfully submitted,



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ee162602 5/16/03

EXHIBIT A

1. Kalunian KC et al. "Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial," *Arthritis Rheum* 2002 Dec; 46(12): 3251-8.

2. Dumont FJ. "IDEC-131. IDEC/Eisai," *Curr Opin Investig Drugs* 2002 May; 3(5):725-34.

3. Boon L. et al. "Preclinical assessment of anti-CD40 Mab 5D12 in cynomolgus monkeys," *Toxicology* 2002 may 15; 174(1):53-65.

4. Elster EA, et al. "Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates," *Transplantation* 2001 Nov 15; 72(9): 1473-8.

5. Boon L., et al. "Prevention of experimental autoimmune encephalomyelitis in the common marmoset (Callithrix jacchus) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses," *J Immunol* 2001 Sept 1; 166(5);2942-9.

6. Brams P. et al. "A humanized anti-human CD1254 monoclonal antibody blocks CD154-CD40 mediated human B cell activation," *Int Immunopharmacol* 2001 Feb; 1(2):277-94.

7. Gobburu JV et al. "Pharmacokinetics/dynamics of 5c8, a monoclonal antibody to CD154 (CD40 ligand) suppression of an immune response in monkeys," *J Pharmacol Exp Ther* 1998 Aug: 286(2);925-30.

8. Kirk Ad et al. "CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates," *Proc Natl Acad Sci U.S.A.* 1997 Aug 5;94(16):8789-94.

9. Davis JC Jr., et al. "Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus," *J Rheumatol* 2001 Han; 28(1):95-101.

☐ 1: Arthritis Rheum 2002 Dec;46(12):3251-8 Related Articles, Links

Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial.

Kalunian KC, Davis JC Jr, Merrill JT, Totoritis MC, Wofsy D; IDEC-131 Lupus Study Group.

University of California, Los Angeles School of Medicine, USA. kkalunian@mednet.ucla.edu

OBJECTIVE: To evaluate the safety and efficacy of a humanized monoclonal antibody against CD154 (IDEC-131) in patients with active systemic lupus erythematosus (SLE). METHODS: In this phase II, double-blind, placebo-controlled, multiple-center, multiple-dose study, 85 patients with mild-to-moderately active SLE were randomized to receive 6 infusions of IDEC-131, ranging from 2.5 mg/kg to 10.0 mg/kg, or placebo over 16 weeks. Efficacy was assessed at week 20, primarily by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and secondarily, by multiple measures of disease activity. Safety was assessed through week 28 by clinical and laboratory evaluation. Immunogenicity studies were also performed. RESULTS: SLEDAI scores improved from the baseline levels of disease activity in all groups, including the placebo group. However, these scores were not statistically different among the IDEC-131 treatment and placebo groups at week 20. Evaluations of secondary variables did not indicate significant differences between the IDEC-131 treatment and placebo groups. The type and frequency of adverse events were similar between the IDEC-131 and placebo groups. CONCLUSION: IDEC-131 administered at doses ranging 2.5-10.0 mg/kg over 16 weeks was safe and well tolerated in patients with SLE. Efficacy of the drug compared with placebo was not demonstrated. There were statistically significant improvements from baseline in all groups, including the placebo group.

Publication Types:
- Clinical Trial

1: Curr Opin Investig Drugs 2002 May;3(5):725-34

IDEC-131. IDEC/Eisai.

Dumont FJ.

Merck Research Laboratories, Department of Immunology, Rahway, NJ 07065, USA. francis_dumont@merck.com

IDEC, in collaboration with Eisai, is developing IDEC-131 (E6040), a humanized monoclonal antibody (mAb) against CD154, the ligand for CD40 also called CD40L or gp39, for the potential treatment of several autoimmune diseases. IDEC-131 is based on technology that IDEC licensed from Dartmouth Medical School where researchers demonstrated the biological effects of the anti-CD154 antibody in animal models of autoimmunity. In January 2001, phase II trials in psoriasis and idiopathic thrombocytopenic purpura (ITP) were initiated. By january 2002, a phase II trial in Crohn's disease was also ongoing. A pilot, multicenter, multiple-dose phase I trial in moderate-to-severe psoriasis was initiated in January 2001. This trial was ongoing in January 2002. IDEC, in collaboration with Dartmouth Medical School had also initiated a phase I trial in multiple sclerosis by March 1999. IDEC-131 was also previously being developed for systemic lupus ezythematosus (SLE), although no further development for this indication has been reported since the disclosure of disappointing phase II results in April 2000. Analysts at Morgan Stanley predicted in February 2002, that the product would be launched in 2005, with sales of US $25 million, rising to US $75 million in 2006.

Publication Types:
- Review
- Review, Tutorial

PMID: 12090546 [PubMed - indexed for MEDLINE]

1: Toxicology 2002 May 15;174(1):53-65

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Preclinical assessment of anti-CD40 Mab 5D12 in cynomolgus monkeys.

Boon L, Laman JD, Ortiz-Buijsse A, den Hartog MT, Hoffenberg S, Liu P, Shiau F, de Boer M.

Tanox Pharma B.V., Sandinostraat 9, 1069 NJ, Amsterdam, The Netherlands.

Monoclonal antibody (Mab) 5D12 is a potent antagonist of the CD40-CD40L pathway. This cellular interaction has been validated in a large number of experimental animal models where dys-regulation of the immune system plays a role. Chimeric 5D12 (ch5D12) was constructed to reduce the potential immunogenicity and enhance the in vivo half-life when used in humans. ch5D12 is a molecularly engineered human IgG(4) antibody containing the variable domains of the heavy and light chains of the murine version of 5D12 (mu5D12). This new chimeric Mab was tested in a marmoset experimental autoimmune encephalomyelitis model and was shown to effectively prevent disease symptoms. The results of this in vivo evaluation supported clinical use of ch5D12 for immune targeted diseases. Therefore GMP material was prepared and a GLP-compliant tissue cross-reactivity study on human tissues (3 donors/37 tissues) and cynomolgus tissues (2 donors/37 tissues) was performed. ch5D12 stained on the surface of B cells and selected dendritic cells and no unexpected cross-reactivity was observed. The identical staining patterns in human and cynomolgus tissues justified the use of cynomolgus monkeys as a relevant model for humans. A GLP-compliant safety and tolerability evaluation for ch5D12 in cynomolgus monkeys was performed using the GMP produced material. Weekly administration of ch5D12 at two dose levels for 4 weeks was shown to be safe and without any side-effects in all monkeys.

PMID: 11972992 [PubMed - indexed for MEDLINE]

1: Transplantation 2001 Nov 15;72(9):1473-8

LWWonline Lippincott Williams & Wilkins

Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates.

Elster EA, Xu H, Tadaki DK, Montgomery S, Burkly LC, Berning JD, Baumgartner RE, Cruzata F, Marx R, Harlan DM, Kirk AD.

NIDDK/Navy Transplantation and Autoimmunity Branch, Bethesda, MD 20889, USA.

BACKGROUND: Allogeneic skin transplantation remains a rigorous test of any immune intervention designed to prevent allograft rejection. To date, no single, clinically available immunosuppressant has been reported to induce long-term primary skin allograft survival in primates. We have previously shown that treatment with the humanized CD154-specific monoclonal antibody, humanized 5C8 (hu5C8), induces long-term renal allograft survival in nonhuman primates. In this study, we evaluated the efficacy of hu5C8 in preventing primary skin allograft rejection in rhesus monkeys. METHODS: Ten rhesus monkeys were transplanted with full-thickness skin allografts mismatched at both class I and class II major histocompatibility loci. Of these, two were given no treatment, five were treated with hu5C8 alone, and three received hu5C8 combined with whole blood donor-specific transfusion (DST). All recipients also received skin autografts for comparison. Animals were followed by inspection, serial biopsy, mixed lymphocyte culture, and alloantibody determination. RESULTS: Treatment with hu5C8 alone or hu5C8 plus DST greatly prolonged allograft survival. Rejection occurred in the untreated group within 7 days. Mean allograft survival in the monotherapy hu5C8 group was >236 days and in the DST group was >202 days; these differences were not significant. Rejection eventually occurred in most animals. Allograft survival was not correlated with the development of T cell hyporesponsiveness in mixed lymphocyte culture. Rejection was not predicted by the development of donor-specific alloantibody. CONCLUSION: These results show that treatment with the CD154-specific monoclonal antibody, hu5C8, greatly delays the onset of acute skin

1: J Immunol 2001 Sep 1;167(5):2942-9

Prevention of experimental autoimmune encephalomyelitis in the common marmoset (Callithrix jacchus) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses.

Boon L, Brok HP, Bauer J, Ortiz-Buijsse A, Schellekens MM, Ramdien-Murli S, Blezer E, van Meurs M, Ceuppens J, de Boer M, 't Hart BA, Laman JD.

Tanox Pharma B.V., Amsterdam, The Netherlands.

Inhibition of CD40-CD40 ligand interaction is a potentially effective approach for treatment of autoimmune diseases, such as multiple sclerosis. We have investigated this concept with a chimeric antagonist anti-human CD40 mAb (ch5D12) in the marmoset monkey experimental autoimmune encephalomyelitis (EAE) model. Marmosets were immunized with recombinant human myelin oligodendrocyte glycoprotein (rMOG) and treated from the day before immunization (day -1) until day 50 with either ch5D12 (5 mg/kg every 2-4 days) or placebo. On day 41 after the induction of EAE, four of four placebo-treated monkeys had developed severe clinical EAE, whereas all animals from the ch5D12-treated group were completely free of disease symptoms. High serum levels of ch5D12 associated with complete coating of CD40 on circulating B cells were found. At necropsy placebo- and ch5D12-treated animals showed similar MOG-specific lymphoproliferative responses in vitro, but ch5D12 treatment resulted in strongly reduced anti-MOG IgM Ab responses and delayed anti-MOG IgG responses. Most importantly, treatment with ch5D12 prevented intramolecular spreading of epitope recognition. Postmortem magnetic resonance imaging and immunohistologic analysis of the CNS showed a markedly reduced lesion load after ch5D12 treatment. In conclusion, the strong reduction of clinical, pathological, and radiological aspects of EAE by ch5D12 treatment in this preclinical model points to a therapeutic potential of this engineered antagonist anti-CD40 mAb for multiple sclerosis.

1: Int Immunopharmacol 2001 Feb;1(2):277-94 Related Articles, Links

A humanized anti-human CD154 monoclonal antibody blocks CD154-CD40 mediated human B cell activation.

Brams P, Black A, Padlan EA, Hariharan K, Leonard J, Chambers-Slater K, Noelle RJ, Newman R.

IDEC Pharmaceuticals Corporation, San Diego, CA 92121, USA.

Humanized anti-CD154 antibody, IDEC-131, had a slightly, but reproducibly, better binding affinity for CD154 (Kd = 5.6 nM), compared to the parent antibody 24-31 (Kd = 8.5 nM). Otherwise it was indistinguishable from the murine parent antibody in its ability to bind to CD154, block CD154 binding to CD40 and inhibit T cell-dependent B cell differentiation. The latter activity was independent of FcR binding as the Fab'1 fragment of IDEC-131 had an equivalent biological activity to that of the whole antibody. IDEC-131 blocked soluble CD154 from inducing proliferation of purified B cells, and blocked T cell dependent anti-tetanus toxoid specific antibody production by human B cells in vitro. IDEC-131, gamma1, kappa, had strong Fc gammaRI, Fc gammaRII and C1q binding, but was unable to induce complement dependent (CDC) or antibody dependent cell-cytotoxicity (ADCC) of activated peripheral blood T cells, which express relatively low levels of CD154. IDEC-131 antibody inhibited both primary and secondary antibody responses to ovalbumin in cynomolgus monkeys at a dose of 5 mg/kg. In non-immunized animals, treatment with IDEC-131 at 50 mg/kg weekly for 13 weeks induced no change in any of the measured lymphocyte subsets, including B cells, CD4+ and CD8+ T cells. Similarly, a safety study in chimpanzees showed no discernible safety related issues at 20 mg/kg, including B and T cell subsets. These results show that the humanized anti-CD154 antibody, IDEC-131, has retained the affinity and functional activity of its murine parent antibody, is unlikely to deplete CD154 positive lymphocytes in humans, and is safe and effective in blocking antibody production in monkeys. Based on its safety and efficacy profile, IDEC-131 is being developed for therapy of systemic lupus erythematosus.

PMID: 11360929 [PubMed - indexed for MEDLINE]

1: J Pharmacol Exp Ther 1998 Aug;286(2):925-30

Pharmacokinetics/dynamics of 5c8, a monoclonal antibody to CD154 (CD40 ligand) suppression of an immune response in monkeys.

Gobburu JV, Tenhoor C, Rogge MC, Frazier DE Jr, Thomas D, Benjamin C, Hess DM, Jusko WJ.

Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14260, USA.

The pharmacokinetics and pharmacodynamics (PK/PD) of chimeric (Ch5c8) and humanized (Hu5c8) 5c8, a monoclonal antibody that binds CD154 (CD40 ligand), thus blocking the interaction between CD40 and CD154, were investigated in cynomolgus monkeys. Single-dose groups (n = 3 animals per dose) received saline, 0.2, 1, 5 or 20 mg/kg i.v. doses of Hu5c8. The repeat-dose groups (n = 4 animals) received 0 or 5 mg/kg i.v. doses of Ch5c8 or Hu5c8 on days 1, 2, 3, 5, 7 and 9. The single-dose PK parameters showed dose proportionality, with a terminal half-life of 300 h, a volume of distribution at steady state of 73 ml/kg and clearance of 0.2 ml.h-1.kg-1. The repeat-dose regimen produced a longer terminal half-life (500 h) and lower clearance (0.13 ml.h-1.kg-1) than in the single-dose groups. The antibody titer to tetanus toxoid (ATT) challenge served as the immunodynamic marker. The primary ATT response consisted of a latent phase of approximately 10 days, during which the immune system was processing antigen but not yet producing antibody, a rise to an antibody maximum titer at approximately 18 days and a decline toward baseline by approximately 40 days in controls. The 5c8 produced a log(dose)-proportional reduction in the area under the curve of ATT. An indirect PK/PD model based on the kinetics of tetanus toxoid exposure and inhibition of ATT production in relation to 5c8 concentrations was developed. A median inhibitory concentration of 0.84 microg/ml and a efficacy of 0.84 reflected marked inhibition of ATT response by 5c8. The model provides quantitation of reduced ATT responses after 5c8 and was applicable to primary and secondary immune responses and to both single-dose and multiple-dose treatments. The monoclonal antibody 5c8 blocks

the CD40 and CD154 interaction, producing consistent and substantive reduction in antibody formation after administration of tetanus toxoid, which can be characterized with PK/PD modeling. It is anticipated that 5c8 may have utility in the treatment of antibody-mediated autoimmune disease.

PMID: 9694951 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A 1997 Aug 5;94(16):8789-94 Related Articles, Links

CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates.

Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH Jr, Knechtle SJ.

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Selective inhibition of T cell costimulation using the B7-specific fusion protein CTLA4-Ig has been shown to induce long-term allograft survival in rodents. Antibodies preventing the interaction between CD40 and its T cell-based ligand CD154 (CD40L) have been shown in rodents to act synergistically with CTLA4-Ig. It has thus been hypothesized that these agents might be capable of inducing long-term acceptance of allografted tissues in primates. To test this hypothesis in a relevant preclinical model, CTLA4-Ig and the CD40L-specific monoclonal antibody 5C8 were tested in rhesus monkeys. Both agents effectively inhibited rhesus mixed lymphocyte reactions, but the combination was 100 times more effective than either drug alone. Renal allografts were transplanted into nephectomized rhesus monkeys shown to be disparate at major histocompatibility complex class I and class II loci. Control animals rejected in 5-8 days. Brief induction doses of CTLA4-Ig or 5C8 alone significantly prolonged rejection-free survival (20-98 days). Two of four animals treated with both agents experienced extended (>150 days) rejection-free allograft survival. Two animals treated with 5C8 alone and one animal treated with both 5C8 and CTLA4-Ig experienced late, biopsy-proven rejection, but a repeat course of their induction regimen successfully restored normal graft function. Neither drug affected peripheral T cell or B cell counts. There were no clinically evident side effects or rejections during treatment. We conclude that CTLA4-Ig and 5C8 can both prevent and reverse acute allograft rejection, significantly prolonging the survival of major histocompatibility complex-mismatched renal allografts in primates without the need for chronic immunosuppression.

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Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus.

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OBJECTIVE: To investigate the safety and pharmacology of a humanized monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus (SLE). METHODS: Cohorts of 3 to 5 patients with symptomatic lupus each received 0.05, 0.25, 1.0, 5.0, or 15.0 mg/kg of IDEC-131 as a single intravenous infusion. Patients were followed for 3 months to evaluate toxicity and pharmacokinetics. RESULTS: This phase I, single dose, dose-escalating study was conducted in 23 patients at a single institution. All patients experienced at least 1 adverse event (AE) during a 3 month followup period, although 58 AE in 17 patients were considered possibly or probably related or of unknown relationship to treatment. No dose relationship in the distribution of AE was apparent. No infusion related cytokine-release syndrome was observed; no infusions were interrupted, and all patients completed treatment. Eight mild (grade 1 or 2) infections were reported in 8 patients. All infections were considered unrelated to drug administration and all resolved uneventfully. No patient developed detectable antibodies to IDEC-131. Flow cytometry revealed no apparent treatment related depletion of lymphocyte subsets. Pharmacokinetic analysis indicated that the maximum serum concentration and the area under the concentration curve of IDEC-131 were proportional to the dose administered. At doses between 1.0 and 15.0 mg/kg, the serum half-life ranged from 299 to 320 h. Efficacy was not formally evaluated in this single dose study. CONCLUSION: IDEC-131 (humanized Mab against CD40L) administered in a single intravenous infusion at doses of 0.05-15.0 mg/kg is safe and well tolerated in patients with SLE.